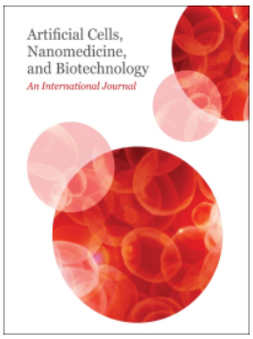

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# Nanogel—an advanced drug delivery tool: Current and future

Ankita Sharma[1], Tarun Garg[1], Amrinder Aman[1], Kushan Panchal[1], Rajiv Sharma[2], Sahil Kumar[2] & Tanmay Markandeywar[1]

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**Abstract**

**Nanogels are robust nanoparticles that could be used to deliver active drug compounds in controlled drug delivery applications. Nanogels drug delivery system is more effective and safer for both hydrophilic and hydrophobic drugs due to their chemical composition and formulations that are inappropriate for other formulations. Nanogels have enabled enlargement of functionalized nanoparticles, which act as a drug carriers that can be loaded with drugs and other active material to be released in a controlled manner at specific site. This review aims at providing general introduction on nanogels, recent synthesis methodology and their novel application in different fields.**

**Keywords:** controlled release, drug delivery, nanogels, polymerization

## Introduction

Nanogels are nanoparticles composed of a hydrogel with cross-linked hydrophilic polymer with 100–200 nm particle size (Garg et al. 2012b). Physically and chemically cross-linked synthetic polymers (Bencherif et al. 2009) or biopolymers (Kabanov and Vinogradov 2009) constitute nanogels. The pores of nanogels are filled with micromolecules or macromolecules (Lee et al. 2007). Nanogels have swelling and degradation properties with flexible size, large surface area and high water content (Hayashi et al. 2004). Nanogels are used to deliver all biologically active agents and are drugs in a controlled and sustained release manner. Nanogels occur in the form of three-dimensional structures in which drugs, polymers and dispersed phase of liquid can be entrapped (Alvarez-Lorenzo et al. 2011) (Vintiloiu and Leroux 2008). Availability of various polymer systems and ease of alteration of their characteristics have rendered nanogels formulations advantageous.

## Advantages of nanogel

A. Nanogels occur withhave high biocompatibility and biodegradable formulation.
B. Nanogels can be controlled for sustained release of drug from the formulation by the addition of a polymeric network. Polymeric networks also control the particle size of the formulation (Sawada et al. 2011).
C. The free-flowing pearlescent solution of the nanogels is easily dispersed in aqueous media (Oh et al. 2008, Samah et al. 2010, Sawada et al. 2011).
D. Nanogels can be easily administered in parenteral and mucosal administration (Garg et al. 2013).
E. The biggest advantage of nanogels is reduced premature leakage of the drug from the solution (Guerrero-Ramírez et al. 2008).
F. Both hydrophilic and hydrophobic drugs can be formulated in nanogels formulation (Garg and Goyal 2014).

## Limitations of nanogel

A) Nanogels have limited drug-loading efficiency and suboptimal regulation of drug release (Wang and von Recum 2011).
B) Sometimes a strong interaction between drug and polymer decreases the hydrophilicity of the nanogels and causes the structure to collapse, hence irreversibly entrapping the drug molecules and enhancing the hydrophilicity of the nanogel matrix (Kabanov and Alakhov 2002, Vinogradov et al. 2002).
C) Surfactant or monomers present in nano gel may cause adverse effects in the formulation (Garg et al. 2014).

## Classification of nanogels according to their structure

Nanogels are classified on the basis of their structures. Different types of nanogels are simple nanogels (artificial chaperons), hollow nanogels including pH- or temperature-sensitive nanogel, cross-linked core—shell nanogels also used to prepare stimuli-responsive nanogel, hairy cross-linked nanogels, multilayer nanogels and functionalized nanogels. Classification of nanogels according to their structure is given in Table I.

Correspondence: Mr. Tanmay Markandeywar, Assistant Professor, Department of Pharmaceutics, ISF College of Pharmacy, Ferozepur Road, Ghal Kalan, Moga, Punjab 142001, India. Tel: + 09878530306. E-mail: tanmayasm@rediffmail.com

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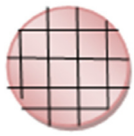
Table I. Classification of nanogel according to their structure.

| S.No | Type | Schematic structure | Network structure | Example |
|---|---|---|---|---|
| 1 | Simple Nanogel |  | a) Cross-linked<br>b) Semi-interpenetrating polymer(semi-IPN)<br>c) Self-assembled | Artificial chaperone, cholesterol-bearing pullulan (CHP) nanogel (Inomoto et al. 2009).<br>Quantum dot nanogel (Wu et al. 2010).<br>Artificial chaperone cholesterol enzymatically synthesized glycogen (CHSEG) nanogel (Takahashi et al. 2010) . |
| 2 | Hollow nanogel |  | Interpenetrating polymer | Stimuli sensitive/responsive nanogel (Xing et al. 2011). |
| 3 | Core—shell nanogels |  | Cross-linked | Stimuli sensitive/responsive nanogel (Sun et al. 2005). |
| 4 | Hairy nanogel |  | Cross-linked | Stimuli-responsive nanogel (Shen et al. 2011). |
| 5 | Multilayer nanogels |  | Cross-linked | Stimuli sensitive/responsive nanogel (Wong et al. 2009). |
| 6 | Functionalized nanogels |  | Cross-linked | Polyethyleneglycol-b-poly (methacrylic acid) [PEG-b-PMA] with PEG terminal aldehyde functionality (Nukolova et al. 2011). |

## Synthesis techniques

### Polymerization of monomers in a homogeneous phase or in a microscale or nanoscale heterogeneous environment

In this method, a colloidal suspension of the polymer is made by the homogeneous nucleation of water-soluble monomers that are used to synthesize stabilized nanogels (Figure 1). This method is especially used to control the particle size. Nanogels of smaller particle size can be synthesized by adding an ionic surfactant, which also increases the colloidal stability of the formulations. As the surfactant concentration decreases, particle size increases in the nanogel formulation (Nayak and Lyon 2005). Donini and coworkers used the precipitation polymerization method to synthesize (PEG)-grafted poly (methacrylic acid) (PMA) nanosuspension in aqueous media. This method is only used for the hydrophobic and thermostable substances and cannot be used for biological molecules (Donini et al. 2002). Some studies included monomer polymerization by inverse microemulsion (w/o) method with the addition of cross-linkers to produce a stabilized nano network (Figure 2). First copolymerization of monomer solubilized in reverse micelles was carried out by Luisi and Straub (Luisi and Straub 1984). Water-soluble nanoparticles are synthesized by several polymers such as polyacrylic acid (PAA) (Kriwet et al. 1998), poly (2-hydroxyethyl methacrylate (PHEMA) (Landfester et al. 2000), and polyacrylamide (PAAm) (Landfester et al. 2000). This method is also used

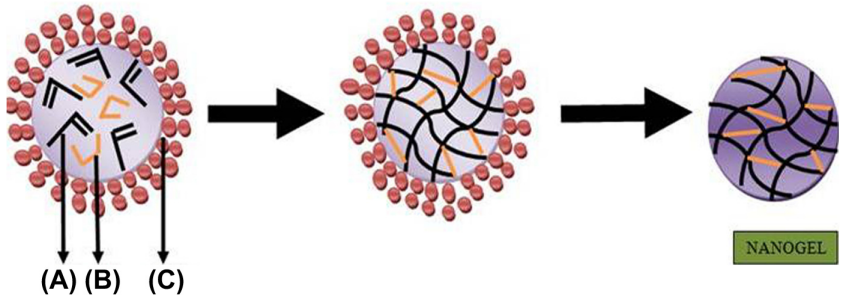



Figure 1. Synthesis of nanogels by copolymerization in colloidal environments. Copolymerization of monomers [A] and bifunctional cross-linkers [B] in w/o microemulsions stabilized by surfactants [C] produces nanogels which can be then transferred into aqueous media after removal of surfactants and organic solvent.

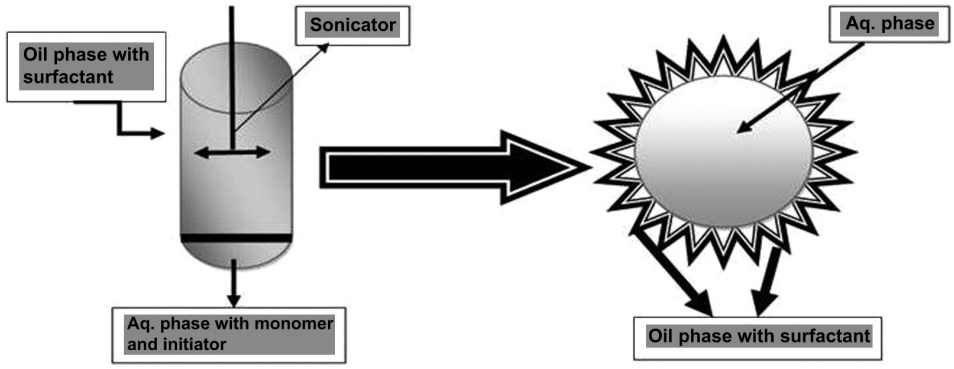



Figure 2. Inverse micro emulsion process.

to synthesize pH-sensitive diethylaminoethyl methacrylate nanogels used for controlled drug delivery system (Marek et al. 2010). In inverse microemulsion method, atom transfer radical polymerization (ATRP) is used to synthesize stable cross-linked nanogels (Oh et al. 2006). Many disulfide cross-linkers were used to synthesize nanogels that are stable in extracellular environment and provide easy release of the drug. Biodegradable nanogels can synthesized by poly [oligo (ethylene oxide)-methyl methacrylate] polymer (Oh et al. 2006).

### Physical self-assembly of interactive polymers

Nanogels are formulated using the physical self-assembled polymers method with amphiphilic polymers, where interaction between the drug and solvent occurs by the Van der Waals' interaction, hydrogen bonding, etc. Micro- and macromolecules are entrapped within the nanogels' structure during self-assembly (Booth and Attwood 2000, Riess 2003). Different protein-loaded nanogels are prepared by self-associating hydrophilic polymers. For example, Akiyoshi et al. prepared insulin hydrogels by the cholesterol-modified pullulan method, where they obtained 20–30 nm particle size. In this method, the size of the nanogels are controlled by proper concentration of polymers and different environmental conditions, such as pH, ionic strength, and temperature (Akiyoshi et al. 1998) (Figure 3). For example, Yu et al. (2006) prepared oppositely charged protein (ovalbumin and lysozyme or ovotransferrin) nanogels by temperature-induced gelation method. Chitosan or ovalbumin can be used to prepare nanogel in the pH- and temperature-induced gelation method. Self-assembled nanogels (120–150 nm) are also prepared using different concentrations of two polymers that are more stable and also suitable for the long-term storage. Amphiphilic block copolymers (hydrophilic and hydrophobic polymers) are widely used to synthesize nanogels in the self-associating polymerization method. Amphiphilic polymers are synthesized by the living radical polymerization (LRP) techniques (e.g., reversible addition–fragmentation chain transfer [RAFT], and nitroxide-mediated polymerization [NMP] methods). Micellization of amphiphilic polymers structure can be modified by changing their nature, length, and composition (Booth and Attwood 2000, Zamurovic et al. 2007). The RAFT process is a single-step synthesis of PEGylated poly (*N*, *N*′-dimethylaminomethyl methacrylate) nanogel using an amphiphilic macro-RAFT agent tri-thiocarbonate with hydrophobic do-decyl chain supporting polymerization (Figure 4). The RAFT method is generally used for gene delivery system, because this method produces smaller particle size, which was appropriate for the gene delivery system (Yan and Tao 2010). Changes in temperature or addition of solvent or additives provide extra degree of freedom to tune the micellar behavior of the amphiphilic block copolymers (Castro et al. 2008, Denkova et al. 2008, Lin and Alexandridis 2002). Surface chemistry of nanogels can be modified for specific tissue and cell targeting. The functionalities of the core of nanogels can be tuned using specific chemical approaches to improve the drug-loading capacity.

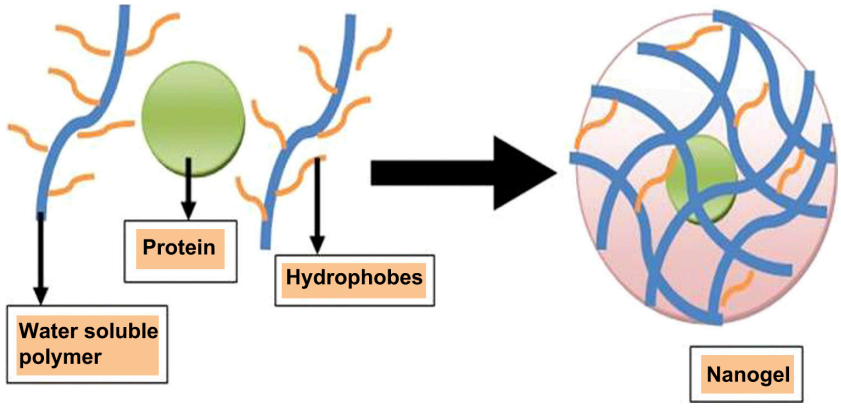



Figure 3. Aggregation of hydrophobically modified polymer, cholesterol-pullulan, in the presence of insulin molecules results in nanogels containing entrapped protein.

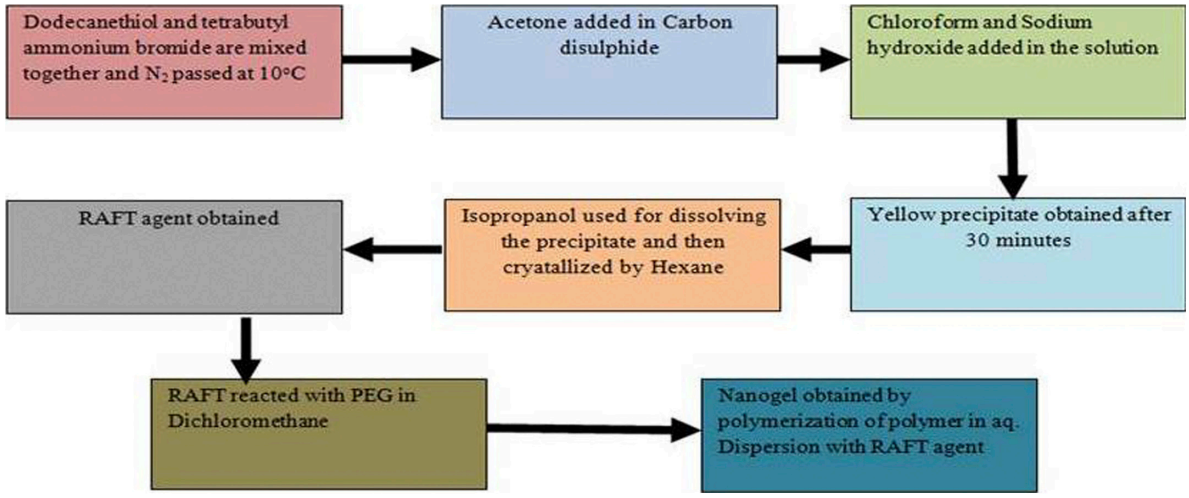



Figure 4. Schematic presentation of RAFT process.

### Cross-linking of preformed polymers

This method is especially used for preparing large-particle-size nanogels. Cross-linked cationic nanogel was used for polynucleotide delivery (Kohli et al. 2007). In cross-linking polymerization method, nanogel was prepared by oil-in-water emulsion, followed by solvent evaporation method where PEG was conjugated to a branched polyethyleneimine (Sun et al. 2005) in aqueous media (Figure 5). Cationic PEI containing nanogels of 80–200 nm diameters were also obtained by the photo-Fenton reaction in aqueous media (Xu et al. 2007, Sun et al. 2006). Cationic nanogels were prepared by using PEI connected by disulfides linkers (Kohli et al. 2007), and hyaluronic acid (Hayashi et al. 2004) nanogels containing biodegradable disulfide linkages were prepared by the inverse water-in-oil emulsion method (Lee et al. 2007). DNA containing cross-linked nanogels was synthesized by mixing thiol-functionalized six-arm branched PEG and DMSO containing DNA by oxidation process (Mok and Park 2006). Both self-assembled method and cross-linking method provided opportunities to control distribution of polymer in nanogels. Various chemically cross-linked nanoparticles found morphologies with spheres, rods, and toroids (Ma et al. 2001). Covalent cross-linking of preformed polymer chains provides excellent opportunities for producing functional nanogels with large pore sizes for drug delivery (Mok and Park 2006). The cross-linking method is especially applied to control the particle size, shape, composition, and surface characteristics of the nanogels. It also provides complete entrapment of the drug in the formulation. Copolymerization method can be carried out in the presence of vinyl monomers, such as PEG triacrylate, PEG monomethyl ether monomethacrylate, and *p*-hydroxy styrene (Gratton et al. 2007)

### Emulsion photopolymerization process

In the emulsion photopolymerization process, UV method is used to prepare dextran nanogel, where dextran hydroxyethyl methacrylate is emulsified with ABIL EM 90 as emulgent in mineral oil, the product is obtained in (1:1) acetone: hexane solution. General emulsion photopolymerization process is given in Figure 6. Meso-tetraphenylporphinedisulfonate is used as a photosensitizer for the breakage of endosomal membranes to release of genes in the cytoplasm and nuclease (Raemdonck et al. 2010).

### Novel pullulan chemistry modification

A mixture of cholesterol isocynate in dimethyl sulfoxide and pyridine was used to synthesize cholesterol-based pullulan (CHP) nanogel. Pullulan is substituted by 1.4 cholesterol moieties per 100 anhydrous glucoside units. All preparations synthesized by the CHP method should be freeze-dried. The CHP method has been especially known to act as good protein carrier nanogels formulation (Alles et al. 2009). Michael addition reaction was used to modify CHP method where the acrylate group and the thiol group were substituted by polyethylene glycol (Hasegawa et al. 2009). A total of 1.1 units of cholesteryl group per 100 glucose units of

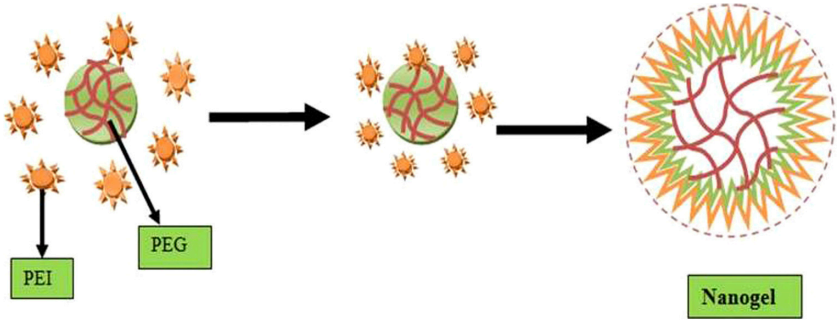



Figure 5. Synthesis of nanogels by cross-linking of the preformed polymer chains or self-assembled polymeric aggregates. Cross-linking of double-end activated PEG and PEI chains in o/w emulsion followed by evaporation of the organic solvent.

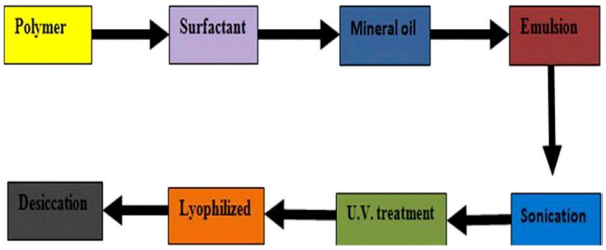



Figure 6. Schematic presentation of emulsion photopolymerisation process.

parent pullulan were used to modify nanosystems of CHP (Figure 7). This modification shows an interaction with Aβ oligomer and monomer for treatment of Alzheimer's disease and also increases microglia and cortical cell viability (Lee et al. 2009). Pullulan was substituted by 1.6 glucose units, which are particularly used for folate receptor targeting. Pullulan and photosensitizer (pheo-A) were coupled with carbodimide and they produced a conjugate, which was converted to nanogel by dialysis. This kind of nanogels is generally used for cancer therapy (Bae and Na 2010).

### Novel photochemical approach

Photochemical approach is used to produce *N*-(2-aminoethyl)methylacrylamide and *N*,*N*′-methylene *bis*-acrylamide-coated ferric oxide nanoparticles nanogels, developed by magnetic resonance imaging (MRI), where nanogels are treated with UV radiation at 388 nm for 10 min (Gong et al. 2009). Similarly plasmid DNA-loaded diacrylated pluronic and glycidyl methyacrylated chito-oligo-saccharide nanogels were prepared at 365 nm UV light with the addition of a photo initiator (Lee et al. 2009).

### Chemical modifications

Chemical modification of polymers supports release of drugs from the nanogels formulation. Acetylation of chondroitin sulfate will increase the release of doxorubicin for 3 weeks in the HeLa cells, which can be used in cancer treatment (Park et al. 2010). Addition of polyion complexes of nanogels consisting of poly 2-(*N*, *N*-diethyl aminoethyl) methacrylate with quaternary group core increases the binding capacity of siRNA for cancer treatment and gene delivery (Tamura et al. 2010). When *N*-isopropylacrylamide and butylacrylate was saturated with sodium carbonate, it altered the drug absorption and release pattern of the Methotrexate (Singka et al. 2010). Modified pH-sensitive hydroxypropylcellulose-polyacrylic acid nanogels are used for bio-imaging of cells by sensing the physicochemical environment. Hydroxyl group of hydroxypropylcellulose-polyacrylic acid was modified by removing cadmium (Hayashi et al.) ions quantum dots and polyacrylic acid to make the formulation pH sensitive and give a better response for cell bio-imaging (Wu et al. 2010). Pluronic nanogels were modified by RNase A with heparin conjugate for easy internalization of the enzyme (Choi et al. 2010). Glycol–chitosan nanogels was grafted by 3-diethylaminopropyl that led to deaggregation of the product at lower pH and also altered the release of doxorubicin (Oh et al. 2010).

## Drug release mechanisms of nanogel

Mechanisms of drug release have been investigated based on the characteristics of the polymer systems such as temperature, pH, and volume transition of nanogels as described further.

### pH-responsive mechanism

Dextran nanoparticles containing nanogel showed on and off catalytic activity for insulin delivery at specific pH. This system is known as glucose-mediated insulin delivery system composed of glucose oxidase, catalase, with chitosan and alginate polymer. The polymer used in the system is insoluble at neutral pH. As pH became acidic, the polymer swells and the drug starts to release from the system (Figure 8). Glucose oxidase is responsible for converting glucose to gluconic acid, which reduces the biological pH (Gu et al. 2013). Owing to the swelling action of pH-sensitive polyacrylic acid chains, Temozolodine showed controlled release kinetics (Wu et al. 2010). The pH-sensitive glycol chitosan nanoparticles (grafted with diethylaminopropyl

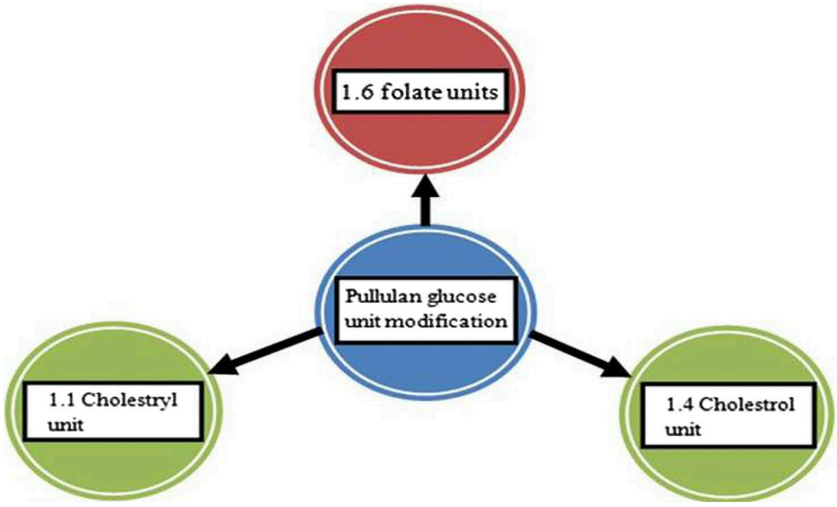



Figure 7. Schematic presentation of pullulan glucose unit modifications.

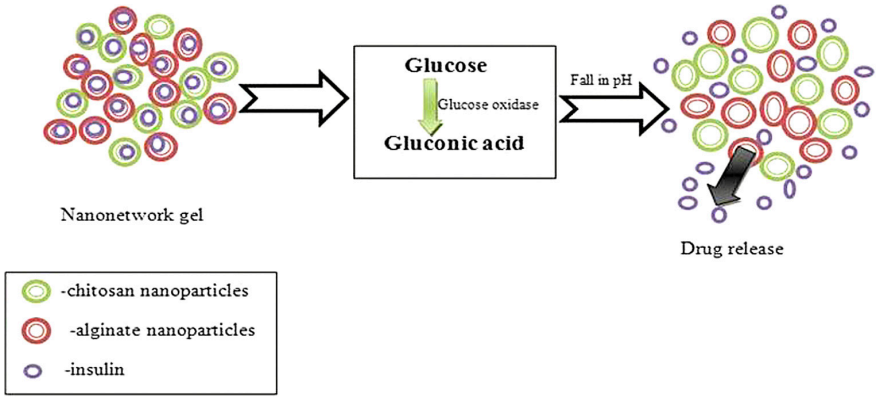



Figure 8. Drug release due to change in pH.

groups) significantly increases the release of Doxorubicin from the formulation (Oh et al. 2010).

### Thermosensitive and volume transition mechanism

Thermosensitive nanogel was developed by poly (*N*-isopropylacrylamide), which releases indomethacin due to maintenance of the temperature above lower critical solution temperature (LCST), which leads to sudden shrinkage in the volume of gel (Figure 9) (Shin et al. 2001). Thermo-responsive nanogel was synthesized by modification of polyethyleneimine with pluronic group, which gives decreased particle size, and was successfully used for gene delivery systems (Lee and Yoo 2008). Thermo-responsive expanded volume poly alkylene oxides nanogel starts to release drug due the destruction of cellular network (Lee et al. 2009). Poly (*N*-isopropylacrylamide) thermosensitive nanogels of doxorubicin are prepared by chitosan-modified chemically reduced graphene oxide (CRGO) process, which is based on near infrared (NIR) method. Graphene used as photo thermal material to respond to NIR, and chitosan is used to prevent aggregation in the formulation of protein. Doxorubicin starts to release drug at 42°C produced by NIR after the shrinkage in poly (*N*-isopropylacrylamide) polymer (Wang et al. 2013).

### Diffusion of the drug from nanogel

Diffusion occurs when a drug or other active agent passes through a polymer that forms a controlled released device. The diffusion can occur on a macroscopic scale as through pores in the polymer matrix or on a molecular level by passing between polymer chains. A normal process of diffusion is given in Figure 10. A polymer and an active agent have been mixed to form a homogeneous system, referred to as a matrix system. In this kind of a system, the combinations of polymer matrices and bioactive agents chosen allow for the drug to diffuse through the pores or macromolecules' structure of the polymer upon introduction of the delivery system into biological environment without including any changes in the polymer itself. The timing of drug release from the delivery system by diffusion can be controlled by two factors: (1) the strength of binding of the drug in the micelle core (e.g., hydrophobic binding in hydrophobic cores), which is characterized by the partitioning of drug between the micelle and external environment, and (2) the polymer chain binding to each other in micelles structure and characterized by cmc (Kabanov et al. 1995). The aforementioned factors show "thermodynamic" and "kinetic stability" of the formulation, which helps to link both drug and micelles and control the drug release (Kabanov and Alakhov 2002). Diffusion of the drug from the smaller size micelle core is usually not rate limiting (for low-molecular-mass drugs). Simple diffusive process involves polymeric release of doxorubicin for a long period and the initial release is controlled by addition of cationic and anionic polyelectrolyte, which increases the size of the nanogel, and doxorubicin starts to release layer by layer without sudden initial outburst (Tan et al. 2008).

## Techniques used to characterize nanogels

### Nanogel formation

Nanogel formation was determined by techniques such as dark field microscopy, NMR studies, RAMAN spectra, and Fourier transform infrared (FTIR).

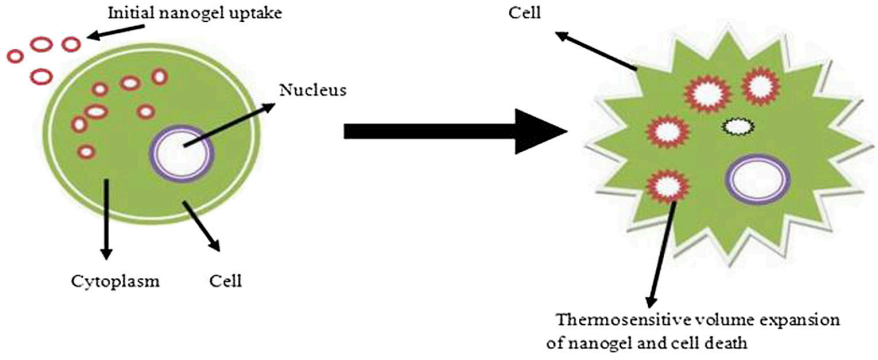



Figure 9. Drug release due to thermo-volume responsiveness of nanogels.

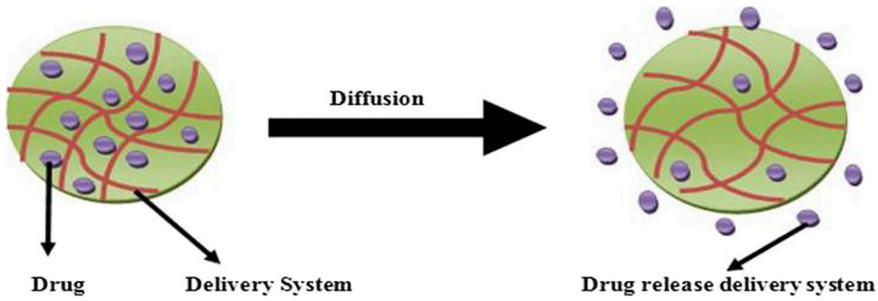



Figure 10. Drug release from nanogel due to diffusion.

### Dark field microscopy

This technique shows the direct image of nanogels within one second after mixing the two polymer solutions. Eclipse E60 Nikon microscope was used to observe the image of β-cyclodextrin polymers (pβ-CD) and dextran (with hydrophobic lauryl side chains) self-assembled nanogels. Both solutions (dextran and pβ-CD) penetrated the capillary and in the little space came in contact with each other and finally reflected as a white spot to give complete formation of nanogel (Gref et al. 2006).

### Nuclear magnetic resonance studies

The magnetic properties of certain atomic nuclei were explained by nuclear magnetic resonance (NMR) technique. Physical and chemical properties of nanogels as well as the structure, reaction state, and chemical environments of the molecule and electronic structure of molecules can be determined by NMR. Aqueous-based cyclodextrin nanogels are characterized by $^{1}$H-NMR and $^{13}$C-NMR spectra, which was recorded with Varian Mercury 300 NMR spectrometer operating at 300 MHz (Kettel et al. 2011). Cationic nanogel was formed by cross-linking between poly (ethylene oxide) (PEO) and poly ethyleneimine (Sun et al. 2005) where the quantity and ratio was determined by $^{1}$H-NMR spectra (Vinogradov et al. 2002). In another example, a self-assembled nano suspension of β- cyclodextrin (β-CD) polymer and dextran with hydrophobic lauryl side chains solution was prepared by the solvent evaporation method. In this nano suspension formulation, β-CD content and substitution in dextran-bearing hydrophobic lauryl side chains solution were determined by $^{1}$H NMR spectroscopy. The $^{1}$H NMR spectra were performed at 25°C on an Inova Varian spectrometer operating at frequencies of 400 MHz, using a 5-mm H X-probe. The spectra were recorded with a flip angle of 90°, a spectral width of 4000 Hz and 256 scans of 16 K points, a repartition time of 15 s at 25, 30, 35, and 40°C (Gref et al. 2006). Inverse microemulsion method was used to develop a pH-sensitive nanogel of *p*-nitro phenol acrylate (NPA) and *N*-isopropylacrylamide (NIPA) in the presence of an aerosol (surfactant) and ethylene glycol dimethacrylate (cross-linking agent). The H$^{1}$NMR spectra show that the copolymer contains both NPA and NIPA monomers. The NMR spectra of nano hydrogels were obtained using a solvent $CDCl_3$ in a Bruker ACE instrument (250 MHz) at 20°C; chemical shifts were measured relative to chloroform ($\iota = 7.26$) (Guerrero-Ramírez et al. 2008).

## RAMAN spectra

It is the shift in wavelength of the inelastically scattered radiation that helps to give information about the chemical and structural data of the formulation. It is used to determine chemical bonds, symmetry of molecules, and crystallographic orientations of a sample. RAMAN spectra is generally used to determine structural information of cyclodextrin-based aqueous nanogel where RAMAN spectra were recorded with a Bruker RFS 100/s (spectral disintegration 4 $cm^{-1}$) (Kettel et al. 2011)

## FTIR techniques

FTIR techniques are used to confirm the structure of the major functional group in the nanogel formulation. Absorption, emission, and photo conductivity of the nanogel were determined by the FTIR method. An FTIR spectrometer collects spectral data in a wide spectral range of the given sample. FTIR spectrophotometer (Nicolet 6700) was used to obtain spectra data of copolymeric nanohydrogels. Guerrero-Ramírez et al. used FTIR to characterize copolymeric nanohydrogel of *p*-nitro phenol acrylate (NPA) and *N*-isopropylacrylamide (NIPA) by inverse microemulsion polymerization method (Guerrero-Ramírez et al. 2008). The nano hydrogels spectrums of FTIR were collected using Attenuated Total Reflectance Smart Orbit accessory. Kettel et al. used FTIR to confirm polymerization in the nanogel of doxorubicin. They also used FTIR for cyclodextrin-based aqueous nanogels. Cyclodextrin content in nanogels has been measured by quantitative analysis of the band at 1032 $cm^{-1.}$ The integrals of this band measured for the nanogel samples were compared with a calibration curve obtained by mixing different amounts of β-CD with KBr (Kettel et al. 2011).

## Structural and morphological determination

### Scanning electron microscope

Scanning electron microscop (SEM) is a type of electron microscopical technique that produces an image of a sample by scanning it with a focused beam of electrons. SEM is able to detect the surface topography of nanogels, composition, three-dimensional structure, chemical composition, crystalline structure, and orientation of the formulation. SEM can achieve a resolution better than 1 nanometer (Kataria et al. 2014). Jayakumar et al. (2012) studied doxorubicin-loaded pH-responsive chitin nanogel for drug delivery; they determine the particle

size and shape by the SEM method. Kettle et al. (2011) also used SEM to determine particle size range of the nanogel.

### Atomic force microscopy

Atomic force microscopy (AFM), also known as Scanning Force Microscopy (SFM), is a very high-resolution type of Scanning Probe Microscopy (SPM). This method is 1000 times superior to the optical diffraction limit. AFM is generally used to determine the size and structure of nanogel. Jayakumar et al. (2012) determined that doxorubicin-loaded chitin nanogels contain spherical shaped nanoparticles. Shen et al. (2011) synthesized biocompatible and thermo-sensitive core–shell nanogel in which AFM was used to determine the size and structure of a nanogel on a Shimadzu SPM-9600 in the tapping mode.

### Transmission electron microscopy

Transmission Electron Microscopy (TEM) is a technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the given sample. A black and white high-resolution image is formed by the TEM when the sample and electrons are interacting. TEMs are able to give information of surface features, shape, size, and structure of the nanogel. Flaws, fractures, and damages occurring in the nanogel formulation can also be determined by TEM. Peng et al. also used TEM to analyze the morphology of dual-responsive cisplatin nanogel (Peng et al. 2013). Gref et al. (2006) determined the structural formation of self-assembled nanogel formed by β-cyclodextrin polymers (pβ-CD) and Dextran bearing hydrophobic lauryl side chains.

## Mean diameter and size distribution determination technique

### Dynamic light scattering

Dynamic light scattering (DLS) is also known as Quasi-Elastic Light Scattering (QELS), or Photon Correlation spectroscopy (PCS). This technique is generally used to determine the size and distribution of the nano molecules. Jayakumar et al. determined the particles size with the help of DLS in a doxorubicin–chitin nanogel (Jayakumar et al. 2012). Shen et al. prepared biocompatible and thermo-sensitive core–shell nanogel that was synthesized by RAFT aqueous dispersion polymerization. They prepared two types of nanogels: one is a linear poly (ethylene glycol) s (*l*-PEGs) and another is a nonlinear analogu as polymers derived from oligo (ethylene glycol) (meth) acrylates (g-PEGs); they are characterized by DLS for their particles structure and size distribution in the absence of surfactant. DLS was performed by a Malvern Zetasizer 3000 HSA at 25°C (Shen et al. 2011). Daoud-Mahammed et al. synthesized nanogel by the spontaneous addition of β-cyclodextrin and modified dextran (modified with alkyl chain) with hydrophobic Benzophenone (guest molecules). In this kind of nanogel, DLS is used to determine the mean diameter and size distribution using a Coulter Nanosizer (Model N4MD, Coultronic, France) (Daoud-Mahammed et al. 2009). Inomoto et al. determined the structure of hydrogel nanoparticles, formed by self-aggregation of cholesterol-bearing pullulan (CHP); this was studied by dynamic light scattering (DLS). DLS measurements were performed by a static/Dynamic Compact Goniometer (DLS/SLS-5000), which determined that a system has unimodel distribution (Inomoto et al. 2009).

### Small-angle neutron scattering

Various structures of the nano substances (size between 1 and100 nm) are investigated by SANS technique. Inomoto et al. determined the structural properties of CHP nanogels and the interaction of CHP nanogel with cyclodextrin, or chaperone-like function by SANS. SANS also shows that, as the concentration of CD increases, the scattering intensity of the CHP nanogel decreases (Inomoto et al. 2009).

## Drug entrapment

### UV spectroscopy

Drug entrapment in nanogel is determined by UV-Vis spectroscopy method. UV-Vis spectroscopy method refers to absorption spectroscopy or reflectance spectroscopy in the Ultraviolet-Visible spectral region (Goyal et al. 2013). Daoud-Mahammed et al. (2009) determined the entrapment efficiency by UV spectroscopy of two hydrophobic molecules (benzophenone and tamoxifen). Wang et al. (2008) also determined the entrapment efficiency by UV spectroscopy method in gelatinized thermo-sensitive nanogels.

## Drug release

### Equilibrium dialysis technique

Equilibrium dialysis is a very simple technique that is used to study the interaction between molecules (Gagandeep et al. 2014). Equilibrium dialysis is an inexpensive method and easy to perform. Nanosized cationic hydrogel was synthesized by the cross-linking between poly(ethylene oxide) (PEO) and polyethyleneimine (Sun et al. 2005). PEO-*cl*-PEI nanogel allowed for immobilization of negatively charged, biologically active compounds such as Retinoic acid, Indomethacin and oligonucleotides or hydrophobic molecules. Release kinetics of the drug from the nanogel was evaluated using the equilibrium dialysis technique, which shows that 17.5% of the drug is released in the first hour and 82% of indomethacin was released in 24 h. This shows that releasing of immobilized biological agent from nanogel system may be useful for the drug delivery system (Vinogradov et al. 2002).

### Franz-diffusion cells

Drug release pattern is also determined by the Franz-diffusion cells (Abu Samah and Heard 2013).

## Swelling ratio

### Equation of Crowther and Vincent

The swelling ratio (Bencherif et al. 2009) of the nanogels was calculated by the equation:

$$\mathrm{SR} = \left[\frac{V_{\mathrm{swollen}}}{V_{\mathrm{shrunken}}}\right] = \left(\frac{D_{20}}{D_{50}}\right)^3$$

Table II. Applications of nanogels in the cancer therapy.

| Nanogel constitution | Type of nanogel | Drug used | Applications | References |
|---|---|---|---|---|
| Maleic acid poly-(*N*-isopropylacrylamide) polymer | Sustained release nanogel/ pH-sensitive nanogel | Doxorubicin | Dual-responsive delivery of Doxorubicin hydrochloride has resulted in low release of drug at a normal physiological pH of 7.4 and temperature 37°C, but a significant release at the cellular pH 4 of cancer cells and a temperature of 41°C | Ballal et al. (2013) |
| Carboxyl group conjugation in nanogel with NaOH used as catalyst | Temperature- and pH-responsive nanogel | Cisplatin | pH thermal dual responsive nanogel mainly used for breast cancer therapy | Peng et al. (2013) |
| Chitin nanogels | Polymerized nanogel | Doxorubicin | This nanogel used for prostate, breast, lungs, liver cancer | Jayakumar et al. (2012) |
| Acetylated chondroitin sulfate | Self-organizing nanogel | Doxorubicin | Used for cancer cell | Park et al. (2010) |
| Hydroxypropylcellulose-poly(acrylic acid) | pH- and temperature-responsive cadmium (Hayashi et al.) ions quantum dots | Temozolomide | Optical pH sensing, cell imaging, and drug loading of Temozolomide | Wu et al. (2010) |
| Glycol chitosan grafted with 3-diethylaminopropyl groups | pH-responsive | Doxorubicin | Doxorubicin uptake accelerated | Singka et al. (2010) |
| Reducible heparin with disulfide linkage | Reducible nanogel | Heparin | Internalization of heparin for apoptotic death of melanoma cells | Bae et al. (2008) |
| Cross-linked folic acid with pullulan | Self-organized with minimal fabrication nanogel | Doxorubicin | Doxorubicin targeting | Kim et al. (2008) |
| Poly(*N*-isopropylacrylamide-*co*-acrylamide) | *In situ* gelatinized thermosensitivenanogel | 5-Flurouracil | Drug-loading capacity of low-mol.-weight 5-Flourouracil was higher than that of biomacromolecules and bovine serum albumin | Wang et al. (2008) |
| Cross-linked Polyethyleneimine and PEG/pluronic | Biodegradable nanogel | 5′-triphosphorylated ribavirin reduced toxicity | | |
| | Biodegradable nanogel | 5-Triphosphate | 5′Triphosphorylated ribavirin reduced toxicity | Kohli et al. (2007) |
| Cross-linked branched network of Polyethyleneimine and PEG | Polyplex nanogel | Fludarabine | Elevated activity and reduced cytotoxicity of Fludarabine | Vinogradov et al. (2005) |

Here, $V_{swollen}$ and $V_{shrunken}$ are the volumes of nanogel particles in swollen status at 20°C and in shrunken status at 50°C, respectively. $D_{20}$ and $D_{50}$ are the average diameters of the nanogels particles at 20°C and 50°C, respectively.

## Stability studies

### Freeze drying

Stability study of biocompatible, thermosensitive core–shell nanogel was done by freeze drying method. Two types of nanogel, *l*-nanogel and g-nanogel, were compared for their ability to survive in freeze-drying condition (Singh et al. 2014, Parnami et al. 2013). The g-nanogels size was unchanged after freeze drying, whereas *l*-nanogels increased in size and polydispersity with freeze drying. Partial crystallization of *l*-PEGs nanogels occurred during freezing and resistant to redispersion of nanoparticles. In contrast, g-PEGs with multiple side chains oligo (ethylene glycol) with a rich collection of conformations, which prevents the crystallization process during freeze-drying condition and thus a better stabilizing effect is achieved (Shen et al. 2011).

### Flocculation in biological medium

Stability of nanogel can also be determined by flocculation method. Aqueous dispersion of core–shell nanogels (*l*-nanogel and g-nanogel) occurred in different medium like 1.5M NaCl solution, 1% bovine serum albumin (BSA), and 100% fetal bovine serum (FBS). Both *l*- and g-nanogels show long-term stability (3 months) in NaCl solution. Both *l*- and g-nanogels shrunk when they transferred from water to NaCl solution due to dehydration. Both *l*- and g-nanogels remained stable in BSA solution for a week but flocculated afterward. In FBS, g-nanogels showed stability more than 4 days, while *l*-nanogels started to aggregate on the third day. All studies show that g-nanogel is more stable than *l*-nanogels (Shen et al. 2011)

### UV-Vis (aminolysis and hydrolysis of the main transfer agents, chain transfer agent CTAs)

Shen et al. (2011) synthesized nanogels (which mainly composed of *l*-PEGs and/or g-PEGs) by the RAFT polymerization method. The stability studies were done by aminolysis and the hydrolysis process of the main transfer agents and chain transfer agent. During the aminolysis process,

Table III. Applications of nanogels in gene delivery, enzymology, and protein folding.

| Nanogel constitution | Type of nanogel | Applications | References |
|---|---|---|---|
| Amphiphilic polysaccharides nanogel with artificial chaperon | Artificial chaperon | Successfully used for the correct folding of Cyclodextrin with rhodanese in cell-free system. Also applied to prevent aggregation of protein | Sasaki et al. (2011) |
| Enzymatically synthesized glycogen synthesized with cholesterol group to<br><br>Enzymatically synthesized glycogen (CHESG) which make nanocomplex with cyclodextrin. | Artificial chaperon | Used for the stabilization of thermal enzyme and also in biomedical engineering | Takahashi et al. (2010) |
| Dextran hydroxyethyl methacrylate-*co*-[2-(methacryloyloxy)-ethyl] trimethylammonium chloride | Nanogels with photochemical internalization | Endosomal escape of siRNA | Raemdonck et al. (2010) |
| Poly [2-(*N*, *N*-diethylaminoethyl) methacrylate] PEGlyated macroRAFT agent. | One-step PEGylated cationic nanogel. | Potential gene therapy | Yan and Tao, (2010) |
| Hybrid hyaluronan hydrogel encapsulating nanogel as a protein nanocarrier | Sustained released nanogel/artificial chaperon nanogel. | Therapeutic peptides and proteins, such as glucagon-like peptide-1, insulin and erythropoietin, were spontaneously trapped in Hyaluronan gel just by immersing hybrid hydrogels into the drug solutions | Hirakura et al. (2010) |
| Cholesterol group bearing pullulan with methyl-β-Cyclodextrin made a complex. | Artificial chaperon/ sustained release | Nanogels did not affect protein synthesis in the cell-free system, providing correctly folded active proteins | Sasaki et al. (2010) |
| Di-acrylated pluronic 127 and glycidyl methacrylated chito-oligosacchride | DNA nanogel with photo cross-linking. | Controlled delivery of plasmid DNA | Lee et al. (2009) |
| Methyl acrylic acid and *N*,*N*'-Methylene-*bis*-(acrylamide) | Super magnetic nanogel functionalized with carboxyl group. | α-Chymotrypsin immobilized on animated Nanogel | Hong et al. (2008) |
| Thiol functionalized Hyaluronic acid | Target specific degradable nanogel | siRNA delivery to HCT-116 cells | Lee et al. (2007) |
| Cholesterol group bearing pullulan | Self-assembled nanogel/ molecular artificial chaperons | This nanogel was an efficient delivery system for bone anabolic agent and cytokines | Nomura et al. (2003) |

g-nanogels were completely aminolyzed at 4°C. A drastic reduction in absorption was seen at 37°C within the first 7 h for *l*-nanogel and complete aminolysis occurred after 52 h. During the aminolysis process, the nanogel sample was colored due to the presence of the CTA, which resulted in hydrolytic stability of the CTAs in the nanogels due to the steric hindrance (Shen et al. 2011).

These results indeed pointed to the enhanced hydrolytic stability of the CTAs in the nanogels due to the steric hindrance. Dithioester group in the g-nanogels in the presence of UV-Vis absorbance show attenuated conditions for a period of 22 days, but the dithioester group in *l*-nanogel started to degrade from the eighth day and a significant decrease (25%) in the absorbance was detected within 22 days.

Table IV. Nanogel applications in gastrointestinal disorder.

| Nanogel constitution | Description | References |
|---|---|---|
| Zwitter ionic poly (carboxybetaine methacrylate) (p-CBMA) nanogels conjugated to cyclo [Arg-Gly-Asp-D-Tyr-Lys] (cRGD) | Nanogels targeted to Human Umbilical Vein Endothelial Cells (HUVECs) in a model cell system where pCBMA was found to selectively bind to HUVECs | Cheng et al. (2010) |

## Thermosensitivity

### $^{1}$H-NMR

Change in the polymer structure is an integral part of temperature responsivity and can be measured by structural characterization techniques known as $H^1$ NMR. A thermosensitivity test is done on doxorubicin nanogels and variation in the intensity or a chemical shift of the characteristic NMR peak in a polymer is determined, which indicates structural variation with different temperature response.

## Applications

### Applications in cancer therapy

Nanogels are used for cancer treatment by incorporating the following drugs: doxorubicin, cisplatin, 5-flurouracil, temozolomide, heparin, etc. Doxorubicin-loaded nanogels are frequently used in the cancer treatment in formulation like pH- and temperature-responsive nanogels in the presence of maleic acid poly-(*N*-isopropylacrylamide) polymer, where doxorubicin is released at a specific pH and temperature. Chitin-polymerized Doxorubicin nanogels are used for treatment of prostate, breast, lungs, and liver cancer (Garg et al. 2012a, Kaur et al. 2014). The different nanogel formulations in cancer therapy are listed below in Table II.

Table V. Marketed formulation of nanogel.

| S.no | Product name | Applications |
|---|---|---|
| 1 | Muc-Off Nano Gel (nanogel bike cleaner concentrate) | It has been specifically formulated to protect disc brake pads and paintwork. It also used removing dirt, sand, traffic film, mud, insects, clay, and oily road grime |
| 2 | Skin perfect brightening nanogel | It brightens the skin and gives complete hydration. It repairs, tones, and protects the skin |
| 3 | NBF Gingival gel | It is a nano-biofusion technology gel. This gel is absorbed in gingival cellular level and maintained with a nano bioactive protecting film. It is a high-quality gum protector |
| 4 | Aqua Multi Effect Nano Gel Cream | It's a moisturizing gel that gives complete hydration for a long time. It also works as anti-wrinkle cream |
| 5 | HA nanogel | Excellent alternative to regular toothpaste. Reducing risk of decay and also reduced bad breath |
| 6 | Zyflex nanogel | It relaxes the muscles and erase the body pain |
| 7 | Oxalginnanogel | It gives deeper action and quicker penetration |
| 8 | Sane care nanogel | Reduces accumulated fats on the abdomen, arms, legs, thighs, and double chin |
| 9 | Revivagenix Pro Collagen Nano Gel | It is an anti-wrinkle cream, gives complete hydration to the skin for longer time |
| 10 | AugenNanogel Eye-care Gel | It is an eye care gel with deep penetration properties |

### Applications of nanogels in gene delivery, enzymology, and protein folding

Nanogel formulations are also used for the delivery of protein, enzymes, and gene at a targeted site. Artificial chaperon is one of the methods that are used to deliver different proteins and enzymes. Modified polymers are used in the artificial chaperon method. Different formulations of nanogels are used in enzymology; protein folding and gene delivery systems are listed below in Table III.

### Nanogel applications in gastrointestinal disorder

Nanogels are also used in gastrointestinal disorders, either in a form of conjugated nanogel or in some other formulation of nanogels (Chang 2007). Nanogel applications in gastrointestinal disorder are listed in Table IV.

## Marketed formulations

Many nanogels formulations are available on the market. Most of the marketed formulations are cosmetic remedies, a number of tooth paste formulation available that reduce tooth decay problems, and several formulations are personal care products that are widely used for skin care problems. A list of various marketed nanogel formulations are given in Table V.

## Conclusion

Nanogel systems have been studied for both theoretical and practical aspects. They are widely used for controlled delivery system, targeted delivery system, coatings purpose, and for the cosmetics products. Nanogels show promising future developments, widening the prospects for drug delivery. Every new analysis entails discovery of recent polymer and mechanistic approaches with a promising role in therapies and innovation on fabrication of nanogels design. The use of nanogels permits the advance of biopharmaceutical parameters of an entrapped drug. We increase the use of these materials in many fields or other delivery systems. Nanogels are probably one of the better drug delivery systems to provided controlled or sustained release of the drug. However, we need to design a perfect delivery system that gives proper knowledge of the interaction between the drug and the carrier and the effect of size and drug loading on drug release.

## Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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